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A Proposed Role for Oxalic Acid in Non-Enzymatic Wood Decay by Brown-Rot Fungi

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This study demonstrates that oxalic acid, which is a fungal secretion product, will reduce ferric iron to the ferrous state at an optimum concentration ratio of 1:1. This reduction of iron significantly increases the cellulolytic activity of the iron-H_2O_2 non-enzymatic decay system. Higher oxalic acid-iron ratios are inhibitory and this is probably due to formation of a 3:1 oxalic acid-iron complex. It was also shown that oxalic acid is a product of the cellulolytic reaction and, hence, may be self-potentiating in this system. Overall, these findings lend support to the hypothesis that the iron-H_2O_2 system is an integral part of the brown-rot decay mechanism. The data in this study were developed as part of a project funded by the Electric Power Research Institute.

Introduction

The fungal decay of wood has generally been considered a purely enzymatic process with the decomposition of cellulose attributed to the activity of the fungal cellulase enzyme complex. The function and synergistic activities of cellulase enzymes are best understood for white-rot basidiomycetes. Culture filtrates of these organisms possess both C_1 and C_x activities (Highley, 1973). The C_1 component is responsible for the modification of highly ordered forms of cellulose while the C_x component is responsible for the hydrolysis of the resultant linear cellulose chains (Cowling and Brown, 1969; King and Vessal, 1968).

Brown-rot fungi apparently lack the C_1 component of the cellulase complex and yet these organisms depolymerize wood cellulose at a faster rate and in a qualitatively different manner than white-rot fungi (Cowling, 1961). Cowling and Brown (1969) have stated that the cell wall capillary structure of wood is too small to allow the migration of cellulases into the wood structure. Thus, the ability of brown-rot fungi to initiate their decay in the secondary cell wall and their rapid, widespread depolymerization of the cellulose does not appear to be attributable solely to an enzymatic mechanism (Bailey, et al., 1968).

Halliwell (1965) showed that a system composed of ferrous salts and hydrogen peroxide (H_2O_2) would decompose cotton cellulose in a manner similar to many cellulolytic microorganisms. He was able to completely solubilize cotton cellulose using physiological concentrations of these reagents in a buffered solution. Cowling and Brown (1969) suggested that such a system might be involved in the brown-rot decay mechanism.

In a series of experiments, Koenigs (1972a, 1972b, 1973, 1974a, 1974b) demonstrated the feasibility of the Fe(II)-H_2O_2 non-enzymatic decay scheme. Brown-rot basidiomycetes were found to produce extracellular H_2O_2 when grown on wood. Koenigs also showed that wood exposed to the Fe(II)-H_2O_2 system possessed many characteristics of brown-rotted wood and was subsequently more susceptible to attack by purified cellulases.

Both Halliwell and Koenigs added exogenous Fe(II) to their systems in achieving their results. Although the level of iron in wood is low (15-19 ppm), the quantity may still be sufficient to increase the susceptibility of wood to enzymatic attack (Koenigs, 1974b; Reese, 1977). However, this iron will most likely be present as Fe(III) which is the most stable valence state. While Fe(III) does react with H_2O_2 to degrade cellulose, the combination yields a much slower and weaker oxidizing system than Fe(II) (Moody, 1964; Buchanan, et al., 1976). Therefore, any organism possessing a non-enzymatic decay mechanism must also be capable of reducing endogenous Fe(III) to Fe(II) (Reese, 1977).

In this regard, Koenigs (1974b) suggested that the low pH maintained by brown-rot fungi may play a role in solubilizing and reducing the endogenous iron found in wood. He demonstrated that high decay isolates of the brown-rot fungi *Lenzites trabea* Pers. ex. Fr. (*Gloeophyllum trabeum*) lowered

the pH of wood, whereas low decay isolates of the same organism actually raised the pH of wood (Koenigs, 1973).

Since oxalic acid is the acid primarily responsible for the maintenance of this low pH (Shimazono, 1955; Takao, 1965), we investigated the possibility that the fungal secretion product, oxalic acid, might have an activator role in the iron-H_2O_2 non-enzymatic decay scheme via the direct reduction of iron.

The reduction of Fe(III) by oxalic acid and its effect on the Fe(III)-H_2O_2 reaction were first examined. The effect of oxalic acid on the decomposition of cellulose by the Fe(III)-H_2O_2 system was then determined along with an examination of the water soluble reaction products.

Materials and Methods

Reagent grade chemicals were used throughout these experiments. Iron was added as either $FeSO_4$ or $Fe_2(SO_4)_3$. Prior to its use, all glassware was rinsed with 0.5 N HCl to avoid any trace iron contamination (Koenigs, 1974b).

Reduction of Iron

The reduction of Fe(III) was determined by measuring the oxidation of oxalic acid according to the following equation:

$$2Fe(III) + HOOCCOOH \rightarrow 2Fe(II) + 2H^+ + 2CO_2 (\uparrow) \quad (I)$$

The evolution of $^{14}CO_2$ was used to measure reaction (I). ^{14}C-Oxalic acid with a specific activity of 4.1 mCi/mmole was obtained from New England Nuclear. All reagents were prepared in a 0.2 M sodium acetate buffer at pH 4.2 previously used by Halliwell (1965). Reactions were carried out at 25°C in a shaker-bath operating at 1 Hz. $^{14}CO_2$ was collected in center wells using a hyamine hydroxide trap. Samples were dark adapted and counted in a Packard Model 3314 liquid scintillation counter using Aquasol LS cocktail. Oxalic acid oxidized to CO_2 was calculated using the following formula:

$$\mu\text{moles oxalic acid oxidized to } CO_2 = \frac{\text{net sample dpm}}{\text{specific activity of oxalic acid}} \quad (II)$$

Oxalic acid concentrations of 0.1, 0.5, 1.0, 5.0, and 10.0 mM were combined with 1.0 mM Fe(III) to give oxalic acid—iron ratios of 1:10, 1:2, 1:1, 5:1, and 10:1. Controls without iron and containing Fe(II) were included.

Effect of Oxalic Acid on the Fe(III)-H_2O_2 Reaction Rate

According to the free radical mechanism proposed by Barb, et al. (1951), the iron-hydrogen peroxide reaction evolves stoichiometric amounts of oxygen in the absence of interfering free radical scavengers. The evolution of oxygen was used to measure the reaction rate of the system under the influence of oxalic acid. A Yellow Springs Instruments Model 53 Biological Oxygen Monitor was used for these determinations and all measurements were made at 30 ± 0.1°C. Reactions were run in acetate buffer (pH 4.2) with iron and H_2O_2 always added to give final concentrations of 1.0 mM and 0.25 percent (73.5 mM), respectively. Oxalic acid concentrations were 0.0, 0.01, 0.1, 1.0, 5.0, and 10.0 mM. Final volume in the reaction chamber was 4.0 ml and oxygen tension was reduced by thorough gassing with nitrogen prior to measurements.

In the first series of experiments, Fe(III) and oxalic acid were allowed to pre-incubate 20 minutes prior to starting the reaction by the addition of H_2O_2. The pre-incubation period was increased to four hours in the second series. Incubations were carried out under nitrogen.

Initial reaction velocity was determined from the slope of the strip chart recordings. Calculations were based on an oxygen concentration of 0.232 μMoles O_2/ml at 100 percent air saturation (Dixon, 1943).

Effect of Oxalic Acid on Cellulose Oxidation

The reaction of the iron-hydrogen peroxide system with carbohydrates yields CO_2 as one of the major products (Moody, 1964). To obtain a very sensitive measurement of this reaction ^{14}C(U)-cellulose was obtained from New England Nuclear at a specific activity of 8.0 μCi/g. The materials and procedures for incubations and assay of the evolved $^{14}CO_2$ were essentially identical to those previously described. Each reaction vessel contained 5.33×10^5 dpm (30 mg) of ^{14}C-cellulose in a final volume of 10 ml.

Fe(III)-H_2O_2 concentrations of 0.1 mM and 0.05 percent, respectively, were prepared with oxalic acid concentrations of 0.0, 0.1 and 1.0 mM. An initial set of experiments used these reagent concentrations for incubations conducted with the standard acetate buffer (pH 4.2). These incubations were then repeated without the buffer using distilled water. $^{14}CO_2$ was collected every 24 hours for three days.

Another series of non-buffered incubations was carried out at Fe(III)-H_2O_2 concentrations of 0.10 mM—0.25 percent and 0.01 mM—0.25 percent, respectively. These incubations were terminated after 24 hours.

Product Evaluation

Following the final collection of $^{14}CO_2$, 0.1 mM Fe(III)—0.25 percent H_2O_2 treated cellulose samples were prepared for thin-layer chromatography by a modification of the procedure used by Blouin and Arthur (1960). Fischer silica gel Redi-plates were used. The solvent systems were:

A. Propanol—ethyl acetate—water (3:2:1)
B. Ethyl acetate—acetic acid—water (3:1:1)
C. Ethyl acetate—pyridine—water (8:2:1)

Chromatograms were visualized using a 0.2 M $AgNO_3$—0.1 N NH_4OH (1:1) spray reagent (Rendina, 1971).

An enzymatic determination was used to test for the presence of oxalic acid as a reaction product. The procedure was based upon the fact that the enzyme oxalic acid decarboxylase is very specific for oxalic acid and catalyzes its decomposition to CO_2 and formic acid. Since any oxalic acid formed in the system from uniformly labelled cellulose will also be labelled, its presence can be detected by the evolution of $^{14}CO_2$. Lyophilized filtrates from ^{14}C-cellulose incubations were reconstituted in 4 ml of a 0.1 M sodium-citrate:HCl buffer at pH 2.7 (Shimazono, 1955). Oxalic acid decarboxylase (Sigma) was prepared in the same buffer. Aliquots of the samples received either enzyme solution or buffer only. One and two hour $^{14}CO_2$ samples were taken using the standard well system. One additional control was run using a warm water wash of ^{14}C-cellulose prior to any incubation.

Results from all experiments were subjected to appropriate statistical analysis.

Results

It is known that the rate of oxidation of cellulose by the iron-H_2O_2 system is dependent on the rate of decomposition of H_2O_2 to the active oxidizing agents, namely, hydroxyl and hydroperoxy radicals. Furthermore, it has been established that H_2O_2 is decomposed much more rapidly in the presence of Fe(II) as compared to Fe(III) (Barb, et al., 1951; Buchanan, et al., 1976). Consequently, because the iron naturally present in wood is in the trivalent state, the role of oxalic acid in accelerating the oxidation of cellulose must be its capability to reduce Fe(III) to Fe(II).

Before proceeding with the effect of oxalic acid on the oxidation rate of cellulose by the iron-H_2O_2 system, some preliminary experiments were conducted to determine:

- the optimum reaction conditions for the reduction of iron with oxalic acid
- the effect of oxalic acid on the Fe(III)-H_2O_2 reaction rate.

Equation (I) shows the overall reaction for the reduction of iron by oxalic acid. As can be seen, oxalic acid is oxidized and CO_2 is evolved in the process and this provides a means of measuring the reduction of iron.

The oxidation of oxalic acid by Fe(III) for a 24 hour period as a function of the initial oxalic acid concentration is shown in Figure 1 and clearly demonstrates that this acid is capable of reducing iron. There is a linear increase in reaction rate with increasing oxalic acid concentration up to a 1:1 oxalic acid : Fe(III) ratio. This pattern begins to break down at 5.0 mM oxalic acid with the appearance of inhibition by high oxalic acid at the 10.0 mM concentration.

From this data it appears that the optimum ratio of oxalic acid to Fe(III) for the reduction of Fe(III) is apparently between 1:1 and 5:1 at the concentrations used here. Since oxalic acid is known to form a strong 3:1 complex with iron, the indications of inhibition seen at 5.0 and 10.0 mM oxalic acid in Figure 1 are probably attributable to binding of the iron which may sterically interfere with the reduction of Fe(III). High oxalic acid to iron ratios also inhibited cellulose oxidations by Fe(III) and H_2O_2 which adds additional support to the contention that complexing of the catalytic iron is the basis for this inhibition.

Since the initial experiments established the fact that oxalic acid at certain concentrations would

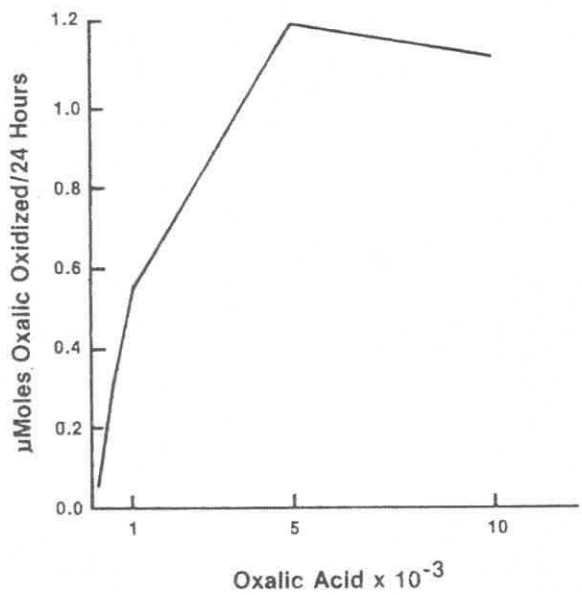

Figure 1.—Oxidation of oxalic acid by 1.0 mM Fe (III) as a function of oxalic acid concentration. Values are expressed as $\bar{X} \pm SE$ for 3 measurements at each concentration.

readily reduce iron, additional studies were conducted to determine its effect on the Fe(III)-H_2O_2 reaction rate. This was done by measuring the rate of evolution of oxygen, which is a product of the iron-H_2O_2 reaction.

Results from the measurements of the iron-H_2O_2 reaction rate were initially discouraging. Comparison of the reaction rate of the Fe(III)-H_2O_2 system with that of the Fe(II) system showed no significant difference. This is in contrast to the literature, which indicates that the Fe(II)-H_2O_2 reaction rate is considerably greater than that observed with Fe(III) (Barb, et al., 1951; Buchanan, et al., 1976).

All determinations started with Fe(II) showed an immediate drop in the oxygen content of the solution upon the addition of H_2O_2, Figure 2. Following this drop the rate of oxygen evolution was comparable to that value obtained for Fe(III). Furthermore, it was also found that the addition of oxalic acid caused a concentration dependent inhibition of the Fe(III)-H_2O_2 reaction. Results from the four hour pre-incubations were similar except at oxalic acid concentrations of 1.0, 5.0, and 10.0 mM. At these concentrations, the addition of H_2O_2 caused an immediate drop in the oxygen content of the solution after which the evolution of oxygen proceeded at a rate characteristic of Fe(III) in the 20 minute pre-incubations with oxalic acid. Figure 2 shows polarographic recordings of these oxygen drops as well as the Fe(III) rate in the absence of oxalic acid.

Explanation of these results requires a closer examination of the entire iron-H_2O_2 reaction scheme (Barb, et al., 1951), which is as follows:

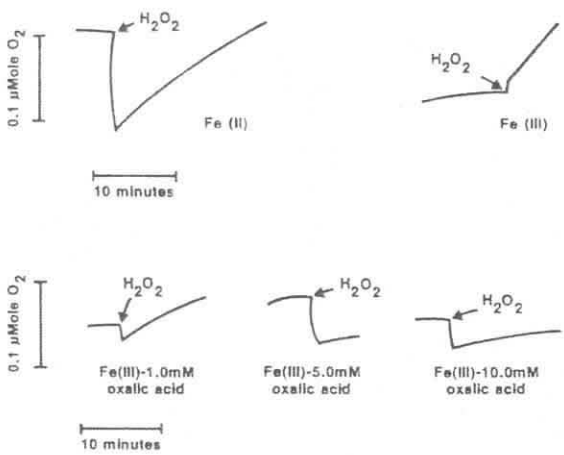

Figure 2.—Polarographic recordings of the iron catalyzed evolution of oxygen from H_2O_2. Iron and H_2O_2 were 1.0 mM and 0.25 percent for all reactions in a 0.2 M acetate buffer, pH 4.2. Iron and iron—oxalic acid solutions were pre-incubated for four hours at 30°C under nitrogen prior to initiating the reaction with H_2O_2.

$$Fe(III) + H_2O_2 \rightarrow Fe(II) + H^+ + HO\dot{O} \text{ (slow)} \quad (III)$$
$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + \dot{HO} \text{ (fast)} \quad (IV)$$
$$\dot{HO} + H_2O_2 \rightarrow H_2O + HO\dot{O} \quad (V)$$
$$Fe(III) + HO\dot{O} \rightarrow Fe(II) + H^+ + O_2 (\uparrow) \quad (VI)$$
$$Fe(II) + HO\dot{O} \rightarrow Fe(III) + HO^-_2 \quad (VII)$$

Reaction (III) is the rate limiting step with its active agent being the hydroperoxy radical. Reaction (IV) is a kinetically fast reaction which yields the highly reactive hydroxyl radical. In the absence of a free radical scavenger, reaction (VII) becomes the chain's terminal step. In the presence of an organic substrate (free radical scavenger) the following reactions interfere with this evolution of oxygen (Buchanan, et al., 1976):

$$\dot{HO} + RH \rightarrow H_2O + \dot{R} \quad (VIII)$$
$$\dot{R} + O_2 \rightarrow RO\dot{O} \quad (IX)$$
$$RO\dot{O} + H^+ + Fe(II) \rightarrow ROOH + Fe(III) \quad (X)$$

An analysis of this reaction scheme and our overall reaction system provides an explanation of the anomalous results discussed above. The important point is that the experimental solutions contained a 0.2 M acetate buffer that provides abundant acetate ions which function as free radical scavengers. Consequently, the sequence of events would be the formation of the hydroxyl radical when H_2O_2 is added in the presence of Fe(II) (reaction IV). Following this the free radical scavenger (acetate ion) would lead to the initiation of reactions (VIII), (IX) and (X). Reaction (IX) is specifically responsible for the oxygen drop observed in our results. These alternate reactions affect both the measured reaction rate through the consumption of oxygen and through actual inhibition of the mechanism by the removal of $\dot{HO}$ and Fe(II) which are chain perpetuating species.

The oxygen drops observed in Figure 2 are, therefore, actually indications of the Fe(II)-H_2O_2 reaction rate. The fact that the rate of oxygen evolution following these drops was characteristic of the Fe(III) system indicated that all available Fe(II) was immediately oxidized. An attempt was made to measure this reaction rate spectrophotometrically based on the absorbence of Fe(III) at 304 nm. Fe(II) and H_2O_2 concentrations used by Koenigs (1974) were tested. In all cases, the lifespan of Fe(II) in the acetate buffer was less than two to three seconds following the addition of H_2O_2.

The apparent inhibition of the iron-H_2O_2 reaction by oxalic acid may also be explained by this mechanism. Since the addition of oxalic acid to a Fe(III) solution leads to Fe(II) production in a concentration dependent manner, it would also lead to the production of the hydroxyl radical at a similar rate (reaction IV). The addition of oxalic acid would therefore cause the eventual removal of oxygen

from the solution (reactions VIII and IX) and give the appearance of a concentration dependent inhibition of the iron-H_2O_2 reaction as measured by oxygen evolution.

Effect of Oxalic Acid on Cellulose Oxidation by the Iron-H_2O_2 System

After it was clearly demonstrated that, under the proper reaction conditions, oxalic acid would accelerate the iron-H_2O_2 reaction, the effect of this acid on the oxidation of cellulose was investigated. The results of both the buffered and non-buffered incubations of ^{14}C-cellulose with 0.1 mM Fe(III)-0.05 percent H_2O_2 at three oxalic acid concentrations are presented in Table 1. At a 1:1 concentration ratio with Fe(III), oxalic acid increased the amount of cellulose oxidized to CO_2 in both buffered and non-buffered systems. The buffered system showed a 52 percent increase above 0.0 mM oxalic acid controls while CO_2 production was increased 93 percent in the non-buffered system. Measurements showed no change in pH due to the addition of 0.1 mM oxalic acid to either system. The additions of oxalic acid at a 10:1 ratio with Fe(III) significantly inhibited both systems to a similar degree.

The greater activity of the Fe(III)-H_2O_2 system in the absence of the acetate buffer is clearly shown in Figure 3 which compares the 0.0 mM oxalic acid controls for both the buffered and non-buffered systems. The amount of cellulose oxidized to CO_2 is increased by an order of magnitude in the 72 hour non-buffered incubations.

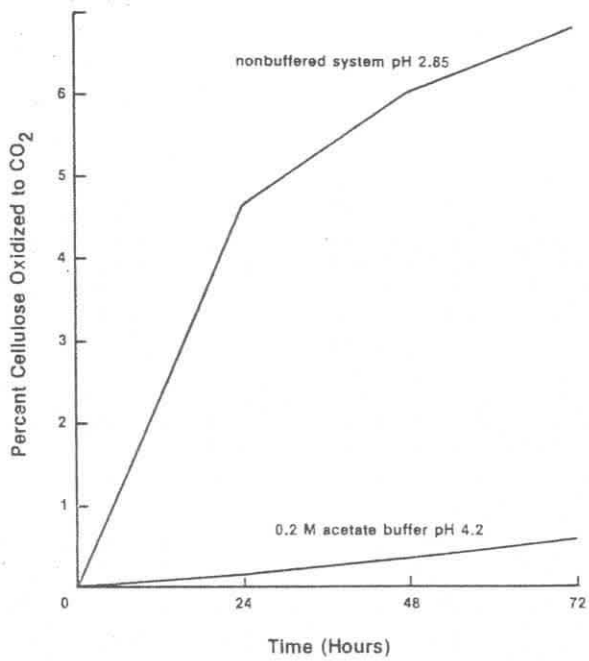

Figure 3.—Comparison of the oxidation rate of ^{14}C-cellulose to $^{14}CO_2$ for buffered and non-buffered incubations at 0.1 mM Fe (III) and 0.05 percent H_2O_2.

Incubations at 0.01 and 0.1 mM Fe(III) with 0.25 percent H_2O_2 again demonstrated that the addition of oxalic acid increased the rate of oxidation of cellulose to CO_2 with the greatest effect at a 1:1 oxalic acid:Fe(III) ratio at 0.01 mM Fe(III) with 0.01 mM oxalic acid, CO_2 production increased 70 percent above 0.0 mM oxalic acid controls as shown in Table 2. The addition of oxalic acid at a 10:1 ratio with iron inhibits the system as previously demonstrated.

Table 1

Percent Oxidation of ^{14}C-Cellulose in Buffered and Non-buffered Solutions

0.2 M Acetate Buffer — 0.1 mM Fe(III) 0.05% H_2O_2

OXALIC ACID	pH	24 HR	48 HR	72 HR	RELATIVE ACTIVITY[1]
0.0 mM	4.2	0.16 ± 0.016	0.36 ± 0.023	0.59 ± 0.008	100
0.1 mM	4.2	0.26 ± 0.039	0.60 ± 0.12	0.90 ± 0.17	152
1.0 mM	4.2	0.05 ± 0.015	0.08 ± 0.016	0.13 ± 0.028	22

Non-buffered — 0.1 mM Fe(III) 0.05% H_2O_2

OXALIC ACID	pH	24 HR	48 HR	72 HR	RELATIVE ACTIVITY[1]
0.0 mM	2.85	4.65 ± 0.06	6.03 ± 0.20	6.82 ± 0.085	100
0.1 mM	2.85	9.20 ± 0.72	11.91 ± 0.72	13.13 ± 0.88	193
1.0 mM	2.50	0.10 ± 0.003	0.13 ± 0.003	0.15 ± 0.004	2

Values are expressed as mean ± SE for three replicates (buffered) and two replicates (unbuffered). All 0.1 mM oxalic acid values were significantly higher and all 1.0 mM oxalic acid values were significantly lower at the 0.05 level compared to 0.0 mM oxalic acid controls using analysis of variance and Student Newman-Keuls tests.

[1]Relative to percent oxidation at 0.0 mM oxalate at 72 hours.

Table 2

Percent Oxidation of ^{14}C-cellulose by 0.1 mM Fe(III) and 0.01 mM Fe(III) with 0.25% H_2O_2 at 24 Hours Non-buffered

OXALIC ACID	0.10 mM Fe(III) 0.25% H_2O_2	RELATIVE ACTIVITY[1]	0.01 mM Fe(III) 0.25% H_2O_2	RELATIVE ACTIVITY[1]
0.0 mM	6.17 ± 0.16	100	1.03 ± 0.10	100
0.01 mM	7.63 ± 0.14	124	1.75 ± 0.47	170
0.10 mM	9.23 ± 0.40	150	0.32 ± 0.11	30

Values are expressed as the mean ± SE for three determinations (0.10 mM Fe(III) or five determinations (0.010 mM Fe(III)). All values were significantly different ($\alpha = 0.05$) within each iron concentration.

[1]Relative to percent oxidation at 0.0 mM oxalate.

Evaluation of Reaction Products

Thin-layer chromatography of the water soluble fraction detected no qualitative differences in the chromatograms of the Fe(III)-H_2O_2 system with or without oxalic acid. Solvents A and B separated 13 components, solvent C yielded 6 spots. Although the complexity of the chromatograms made identification difficult, four components were identified by their migration with standards in at least two of the solvents. Glucose, glucuronolactone, gluconolactone, and oxalic acid were all confirmed as products. Cellobiose and saccharic acid lactone were tentatively identified. The presence of oxalic acid was confirmed by enzymatic determination. Results from duplicate determinations on two samples showed oxalic acid to be present as an oxidative decomposition product of cellulose. Activity in the cellulose wash was comparable to blanks.

Discussion

Both Halliwell and Koenigs reported carrying out incubations of cellulose or wood in Fe(II)-H_2O_2 solutions for several days. Our results indicate that virtually all the Fe(II) added to their systems would have been consumed in the first few seconds of the incubation period. This suggests that the results from their experiments are largely attributable to an Fe(III)-H_2O_2 system since Fe(III) would have rapidly become the principal iron species in solution. The use of an acetate buffer may have also led to an underestimation of the capacity of a non-enzymatic iron-H_2O_2 cellulolytic system. Removal of the acetate buffer accounted for a 25 fold increase in the amount of cellulose oxidized to CO_2 in the first 24 hours (Figure 3). Halliwell also used a phosphate buffer in his investigation; but other work has shown the phosphate ion also inhibits the reaction between iron and H_2O_2 (Schumb, *et al.*, 1955).

The ability of oxalic acid to increase the activity of the Fe(III)-H_2O_2 system was clearly demonstrated. This effect is independent of pH as shown in Table 1, suggesting that the reduction of Fe(III) to the more catalytically active Fe(II) is responsible. The lowest iron concentration tested (0.01 mM) was still approximately 10 fold higher than levels found naturally in wood. However, considering the 70 percent increase in activity observed with oxalic acid at this iron concentration, it seems likely that a similar effect would occur at the iron levels found in wood. In wood where iron is so critically limiting, a 70 to 100 percent increase in the activity of the Fe(III)-H_2O_2 system could make a very important contribution to the overall decay process. Such a system could function in a preparatory way by acting on the pore structure of the wood thus increasing its susceptibility to subsequent enzymatic attack.

The presence of oxalic acid as a product of the iron-H_2O_2 system opens the interesting possibility of a self potentiating system. Although the amounts produced by the system may be small, the low levels of iron available in wood would require only small amounts of oxalic acid for activation. The oxalic acid produced from the decomposition of cellulose could thus conceivably function to reduce more iron and further potentiate the system.

As a result of this study, a hypothetical wood decay scheme for brown-rot fungi, including the proposed role of oxalic acid in the initial non-enzymatic attack, is presented in Figure 4. Three fungal secretions operate in this scheme: oxalic acid, H_2O_2, and the cellulases. Oxalic acid functions as an activator of endogenous iron thus potentiating the cellulolytic activity of the iron-H_2O_2 mechanism. One product of this activity is oxalic acid which may activate more iron. The overall effect would be to increase the susceptibility of the wood structure to the cellulases and could account for some of the qualitative and quantitative differences observed in brown-rot decay when compared with decay caused by white-rot fungi.

Conclusions

The results of this experiment lend additional support to the hypothesis that brown-rot fungi employ a non-enzymatic iron-H_2O_2 system in their overall decay mechanism. Since it was demonstrated that under the proper reaction conditions oxalic acid will significantly accelerate the cellulolytic activity of this system by reducing iron, it may play a key role in this reaction. Furthermore, oxalic acid is one of the products of the cellulolytic reaction which suggests that it may be self-potentiating.

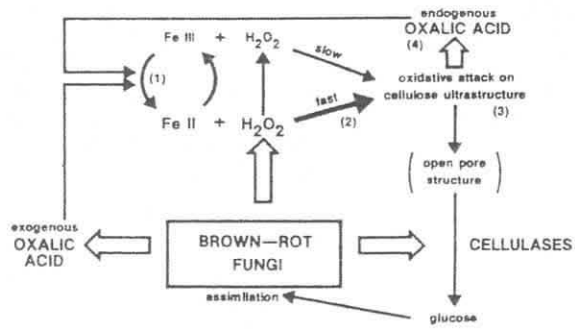

Figure 4.—Hypothetical wood decay scheme for brown-rot fungi including the proposed role of oxalic acid in the initial non-enzymatic attack.

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Discussion

LANCE COLLISTER: Oxalic acid has been used from time to time in the log building industry as an on-site treatment to remove the discoloration of the wood caused by weathering or staining. Am I correct in understanding that the addition of oxalic acid may in actuality be providing a breeding ground for subsequent brown rot growth?

MR. SCHMIDT: The concentration of oxalic acid that give you this effect is a narrow range where this will occur. If you get above, what we believe to be a three to one molor concentration ratio with the iron in the wood, oxalic acid and iron form a very strong complex and this apparently binds the iron up in a nonreactive form in which case the reaction does not go. So adding saturating amounts of oxalic acid to a system might in actuality inhibit the fungus to some degree.

W. H. HARTFORD: Did you ever have a chance to try a buffer such as phosphate where you would be unlikely to form any free radicals as sort of a check against the buffer mechanism.

MR. SCHMIDT: I thought of that and in doing some reviewing of the literature in this regard, I found where someone had done some work with the phosphate buffer and they had shown that phosphate itself is also inhibitory to the reaction, we didn't carry through with that.

B. F. MURPHY: That was an excellent presentation. Why did you only select oxalic acid as was mentioned here for removing iron stains, you have citric acid, tartaric acid and phosphoric acid. Have you investigated those.

MR. SCHMIDT: No we haven't and the main reason we chose oxalic acid was the fact that oxalic acid was the principle acid secreted by these organisms. It was one of the things that early researchers noted and used that to separate the brown rot fungi as a group. They produced various acidic conditions in their decay site which is in contrast to something like a soft rot organism. People traced this down and found it to be largely due to the secretion of oxalic acis so that was the first acid we went to, otherwise, I see your point. It is conceivable that other acids would function in the same way..

PRESIDENT BURDELL: On behalf of the Association I certainly appreciate the work you have done on this excellent paper on the mechanisms and decomposition in specific areas and it is with great pleasure and pride that I give this Graduate Student Award to Christopher J. Schmidt for his research work. Also, Christopher is the fifth recipient of this Award. Just to refresh your memory briefly, 1976—Martins A. Kalinins of FPL Laboratories; 1977—Haryanto Yudoibroto, University of Illinois; 1978—Jorge A. Duran, University of New York; 1979—Abraham Sho-Chein Chen, University of Illinois and now Christopher. We would appreciate it if you would take this back to the University and see how many years you can keep it. Next year we hope that we will have another Student Award. Thank you.

SESSION CHAIRMAN GRAVES: The last paper this morning is by Title Only and is entitled "The Individual Oils of the 1958 Cooperative Creosoted Stake Tests: The Log-Probability Model and the Performance Index" by W. H. Hartford and R. H. Colley. Win Hartford will present the paper.